# Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/043760

International filing date: 22 December 2004 (22.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/531,266
Filing date: 22 December 2003 (22.12.2003)

Date of receipt at the International Bureau: 18 February 2005 (18.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1283374

# THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

*February 09, 2005*

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.**

**APPLICATION NUMBER:** *60/531,266*
**FILING DATE:** *December 22, 2003*
**RELATED PCT APPLICATION NUMBER:** *PCT/US04/43760*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE


# PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

**Express Mail Label No.** _____

| INVENTOR(S) | | |
|---|---|---|
| Given Name (first and middle [if any]) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| PAUL JOSEPH | BOBROWSKI | Scottsdale, ARIZONA |

*Additional inventors are being named on the* _____ *separately numbered sheets attached hereto*

**TITLE OF THE INVENTION (500 characters max)**

METHODS AND COMPOSITIONS TO ENHANCE ENDOGENOUS IGF PRODUCTION AND THEIR USES

*Direct all correspondence to:* **CORRESPONDENCE ADDRESS**

☐ Customer Number: _____

*OR*

| | | | | | |
|---|---|---|---|---|---|
| ☑ Firm or Individual Name | PAUL J. BOBROWSKI | | | | |
| Address | 5030 E Libby Street | | | | |
| Address | | | | | |
| City | Scottsdale | State | AZ | Zip | 85254-7631 |
| Country | USA | Telephone | 602.332.0225 | Fax | 602.795.9528 |



**ENCLOSED APPLICATION PARTS** ***(check all that apply)***

☑ Specification *Number of Pages* 18 ☐ CD(s), Number _____

☑ Drawing(s) *Number of Sheets* 6 ☐ Other (specify) _____

☐ Application Date Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☑ A check or money order is enclosed to cover the filing fees.

☐ The Director is herby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE Amount ($): 80-

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____



*Respectfully submitted,*

SIGNATURE _____

TYPED or PRINTED NAME PAUL J. BOBROWSKI

TELEPHONE 602.332.0225

Date 19 DECEMBER 2003

REGISTRATION NO. _____ *(if appropriate)*

Docket Number: _____

***USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT***

PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE



# FEE TRANSMITTAL for FY 2004

*Effective 10/01/2003. Patent fees are subject to annual revision.*

☑ Applicant claims small entity status. See 37 CFR 1.27

**TOTAL AMOUNT OF PAYMENT** ($) 80 -

| Complete if Known | |
|---|---|
| Application Number | |
| Filing Date | |
| First Named Inventor | PAUL J. BOBROWSKI |
| Examiner Name | |
| Art Unit | |
| Attorney Docket No. | |

## METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account:

Deposit Account Number

Deposit Account Name

**The Director is authorized to:** *(check all that apply)*

☐ Charge fee(s) indicated below ☐ Credit any overpayments

☐ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, **except for the filing fee** to the above-identified deposit account.

## FEE CALCULATION

### 1. BASIC FILING FEE

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1001 | 770 | 2001 | 385 | Utility filing fee | |
| 1002 | 340 | 2002 | 170 | Design filing fee | |
| 1003 | 530 | 2003 | 265 | Plant filing fee | |
| 1004 | 770 | 2004 | 385 | Reissue filing fee | |
| 1005 | 160 | 2005 | 80 | Provisional filing fee | 80.00 |

SUBTOTAL (1) ($) 80 -

### 2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

| | | Extra Claims | | Fee from below | Fee Paid |
|---|---|---|---|---|---|
| Total Claims | | -20** = | | X | = |
| Independent Claims | | - 3** = | | X | = |
| Multiple Dependent | | | | | = |

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description |
|---|---|---|---|---|
| 1202 | 18 | 2202 | 9 | Claims in excess of 20 |
| 1201 | 86 | 2201 | 43 | Independent claims in excess of 3 |
| 1203 | 290 | 2203 | 145 | Multiple dependent claim, if not paid |
| 1204 | 86 | 2204 | 43 | ** Reissue independent claims over original patent |
| 1205 | 18 | 2205 | 9 | ** Reissue claims in excess of 20 and over original patent |

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

## FEE CALCULATION (continued)

### 3. ADDITIONAL FEES

| Large Entity Fee Code | Large Entity Fee ($) | Small Entity Fee Code | Small Entity Fee ($) | Fee Description | Fee Paid |
|---|---|---|---|---|---|
| 1051 | 130 | 2051 | 65 | Surcharge - late filing fee or oath | |
| 1052 | 50 | 2052 | 25 | Surcharge - late provisional filing fee or cover sheet | |
| 1053 | 130 | 1053 | 130 | Non-English specification | |
| 1812 | 2,520 | 1812 | 2,520 | For filing a request for *ex parte* reexamination | |
| 1804 | 920* | 1804 | 920* | Requesting publication of SIR prior to Examiner action | |
| 1805 | 1,840* | 1805 | 1,840* | Requesting publication of SIR after Examiner action | |
| 1251 | 110 | 2251 | 55 | Extension for reply within first month | |
| 1252 | 420 | 2252 | 210 | Extension for reply within second month | |
| 1253 | 950 | 2253 | 475 | Extension for reply within third month | |
| 1254 | 1,480 | 2254 | 740 | Extension for reply within fourth month | |
| 1255 | 2,010 | 2255 | 1,005 | Extension for reply within fifth month | |
| 1401 | 330 | 2401 | 165 | Notice of Appeal | |
| 1402 | 330 | 2402 | 165 | Filing a brief in support of an appeal | |
| 1403 | 290 | 2403 | 145 | Request for oral hearing | |
| 1451 | 1,510 | 1451 | 1,510 | Petition to institute a public use proceeding | |
| 1452 | 110 | 2452 | 55 | Petition to revive - unavoidable | |
| 1453 | 1,330 | 2453 | 665 | Petition to revive - unintentional | |
| 1501 | 1,330 | 2501 | 665 | Utility issue fee (or reissue) | |
| 1502 | 480 | 2502 | 240 | Design issue fee | |
| 1503 | 640 | 2503 | 320 | Plant issue fee | |
| 1460 | 130 | 1460 | 130 | Petitions to the Commissioner | |
| 1807 | 50 | 1807 | 50 | Processing fee under 37 CFR 1.17(q) | |
| 1806 | 180 | 1806 | 180 | Submission of Information Disclosure Stmt | |
| 8021 | 40 | 8021 | 40 | Recording each patent assignment per property (times number of properties) | |
| 1809 | 770 | 2809 | 385 | Filing a submission after final rejection (37 CFR 1.129(a)) | |
| 1810 | 770 | 2810 | 385 | For each additional invention to be examined (37 CFR 1.129(b)) | |
| 1801 | 770 | 2801 | 385 | Request for Continued Examination (RCE) | |
| 1802 | 900 | 1802 | 900 | Request for expedited examination of a design application | |

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($)

| SUBMITTED BY | | | | (Complete *(if applicable)*) | |
|---|---|---|---|---|---|
| Name (Print/Type) | PAUL JOSEPH, BOBROWSKI | Registration No. (Attorney/Agent) | | Telephone | 602.332.0225 |
| Signature | | | | Date | 19 DEC 03 |

**WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**



*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

# METHODS AND COMPOSITIONS TO ENHANCE ENDOGENOUS IGF PRODUCTION AND THEIR USES

## BACKGROUND OF THE INVENTION

### Field of Invention

[001] Aging and certain disease states are associated with a decline in levels of insulin-like growth factor ("IGF"). This deficit leads to morbidity, compromised quality of life, and increased risk for death. While growth hormone primarily regulates the hepatic production of IGF, options to elevate tissue production of IGF are not available.

[002] By contrast, there are a wealth of approaches to reduce IGF production at these sites including hypoxia, aging, cytokines, matrix metalloproteases, infection and heightened immunity.

[003] IGF is highly conserved and serves comparable roles in many species. It is a primary determinant of growth in the young, provides an anabolic effect and enhances muscle tone and strength, as well as maintains and promotes cartilage and bone health and density. IGF functions to regulate the tissue uptake of glucose from whence its name is derived. It is also formed locally within the gonads and promotes fertility and fetal development. IGF promotes the resistance of tissues to stress, toxic and inflammatory challenges.

[004] The challenge to date has been how to promote or restore IGF production in states where IGF can provide a significant benefit. The oral administration of IGF is ineffectual as it is degraded by the digestive system. Similar problems exist with the

oral administration of growth hormone and furthermore, it cannot stimulate local IGF production in many of the desired applications.

[005] To meet this challenge we have evaluated the effects of the Family Brassicaceae vegetable, Maca (*Lepidium* species). Conceptually, Maca is used to treat complications of living at high altitude where it is grown in the Andes and hypoxia has been described to reduce IGF production. Maca has been shown to restore fetal growth compromised by high altitude and IGF is a critical determinant of fetal growth. While Maca has been used as an aphrodisiac, these benefits cannot be explained by a stimulation of testosterone, estrogen, progesterone, prolactin, gonadotrophins or thyroid hormone. To date, the bases for the reproductive stimulatory effects of Maca have not been explained.

[006] Utilizing two new approaches, our research described herein defines the mechanism by which Maca provides benefits. Firstly we demonstrated that Maca is anabolic and growth promoting in farmed fish when Maca or Maca extracts were included in their diets. Secondly, utilizing a cell line that expresses IGF, which has been shown to be suppressed by inflammatory cytokines that promote cachexia, we demonstrated that Maca extracts, particularly polar extracts, directly stimulate IGF gene expression.

[007] Given that suppressed IGF is a critical contributor to the complications and pathology of numerous disease states and the aging process, a new approach that restores IGF levels provides a significant improvement in how these conditions are managed. The methods and compositions described herein concentrate the desired

bioactive constituents with the ability to promote IGF and provide solutions to these conditions.

## SUMMARY OF THE INVENTION

[008] Aspects of the invention are summarized below to aid the understanding of the embodiment of the invention and the application. Yet, the invention is fully described by the claims of the application.

[009] There is a general recognition that insulin-like growth factor (IGF) is an important determinant of growth in young animals. Its production declines with age and in so doing contributes to the symptoms of aging, such as decreased mobility, muscle wasting and strength, diabetes and fertility. The suppression of IGF in these and disease states contributes to the pathology of those conditions. Nevertheless there are limited opportunities to restore or raise IGF levels that are reduced or are inadequate. Growth hormone is a well known stimulus but growth hormone primarily targets hepatic IGF formation with little influence on target tissue production of IGF.

[010] No pharmacological agents or substances have been described to promote endogenous IGF production and gene expression in target tissues. The present invention describes a compositions and methods from a natural source that meets these unmet challenges and in so doing offers a significant innovation to the treatment of these various disorders.

[011] Compositions described herein concentrate the bioactivity, derived from a natural source from the Family Brassicaceae, Maca, and demonstrates a significant and sustained elevation of IGF-1 gene expression in muscle. Related to this we have demonstrated that the parent botanical and polar extracts, but not lipidic

extracts, promote anabolism and growth in fared trout, a bioassay system that is critically dependent on IGF.

[012] Prior art exists for the extraction of Maca. Zheng *et al* (Pat. Nos. 6,267,995, 6,428,824, 6,552,206) report aqueous, agueous:organic and preferably organic methods of extraction for deriving lipidic materials with applications for use in cancer and fertility but primarily sexual dysfunction (*Urology*, 55:598-602, 2000). Additionally, the mechanisms for these purported therapeutic activities are elusive and remain unresolved. However, it is clear that the classic sex steroid, gonatrophins, prolactin and thyroid hormone pathways cannot adequately explain Maca's biological effects.

[013] The present invention describes methods which isolate and concentrate the polar, non-lipidic components of Maca that promote levels of IGF. In comparison, it offers a distinct advantage over the parent botanical and other extraction methods which concentrate lipidic components for other applications. Based upon the current and clear understanding of the role of IGF in aging and pathology, the invention teaches new methods and therapies for addressing associated states.

## BRIEF DESCRIPTION OF THE FIGURES

[014] FIGURE 1: Enhanced expression of IGF-1 in myoblasts (C2C12) cells treated with two different Maca extracts (RNI 510 and RNI 520) at 10ug/ml. Gene expression was determined by RT-PCR at 4 hours. RNI 510, an aqueous extract, was more potent than RNI 520, a methanolic extract.

[015] FIGURE 2: Enhanced expression of IGF-1 in myoblasts (C2C12) cells treated with two different Maca extracts (RNI 510 and RNI 520) at 10ug/ml. Gene expression was determined by RT-PCR at 24 hours. RNI 510, an aqueous extract, was more potent than RNI 520, a methanolic extract. RNI 510 produced a more sustained elevation of IGF-1 gene expression.

[016] FIGURE 3: Effects of Maca supplementation on the growth of Rainbow trout. Maca was administered in the feed at concentrations of 5, 10 and 15%, with growth trout size determined over a 14 week period.

[017] FIGURE 4: The feed conversion ratio, calculated from the dry feed intake divided by the wet body weight gain, in control and Maca feed trout after 14 weeks of testing. In all Maca fed groups (5, 10 and 15%) there was a reduction in the feed conversion ratio indicating a more efficient conversion of food to fish, and an anabolic response.

[018] FIGURE 5: The protein efficiency ratio, calculated from the body weight gain per unit of the protein intake, is significantly enhanced in all Maca fed trout groups (5,10 and 15%). This is indicates a more efficient use of feed sources for growth purposes, or anabolism.

[019] FIGURE 6: Comparison of lipidic and methanolic extracts of Maca on trout growth. Only the Maca control and to a lesser extent the methanolic extract promoted fish growth. This indicates that the biological constituents responsible for growth are likely to be small, non-polar materials, and that extracts that are more polar that methanol would be the most effective.

## DESCRIPTION OF EMBODIMENTS

### Extraction Procedures

[020] According to one aspect of this invention, a process that concentrates polar components optimizes the extraction of Lepidium species ("Maca"), and the Brassicaceae family, to promote the expression of the gene for insulin-like growth factor (IGF) in a manner that is independent of growth hormone. This extraction process concentrates the parent material by at least 75%, independent of lipidic constituents. Preferred methods to accomplish the aforementioned Lepidium species extraction are described by the procedures below but it is contemplated that a skilled practitioner could device obvious variations of the procedures given the disclosure herein and the desired results.

[021] Extraction Process 1

[022] Maca hypocotyls are harvested, cleaned and washed to remove all detritus material and then the hypocotyls macerated. The macerate is then layered onto stainless steel mesh pans and aligned in a rack formation. Steam is then channeled through the mesh pans, the condensate collected and the macerate discarded. The steam may be mixed with a low concentration of an alcohol. The condensate is reduced in volume by various means, including but not limited to evaporation under heat, vacuum or freeze drying. Heating to 75 degrees C produced acceptable results. The resultant material concentrates polar components which stimulate IGF while depleting lipidic and complex starch components.

[023] Extraction Process 2

[024] Maca hypocotyls are harvested and cleansed to remove detritus material as described above. The hypocotyls are then dried and ground into a powder to increase the surface area of the material. This material is then placed on stainless steel mesh sheets, subjected to steam and the condensate collected. The condensate is reduced in volume by various means, including but not limited to evaporation under heat, vacuum or freeze drying. The resultant material concentrates polar components which stimulate IGF while depleting lipidic and complex starch components.

[025] ENHANCED IGF EXPRESSION, PRODUCTION AND APPLICATIONS

[026] The expression of insulin-like growth factor 1 (IGF-1) is a critical determinant of many conditions, where inadequate or decreased production is linked to dysfunction, disease and the symptoms of aging. Hepatic production of IGF can be stimulated by growth hormone but many tissues including skeletal muscle, cartilage, placenta and reproductive organs, produce IGF-1 for autocrine and paracrine reasons. Aging as well as dietary alterations, hypoxia and disease can reduce IGF-1 production but to date other than growth hormone, the ability to promote endogenous (local) IGF-1 production by natural chemicals or pharmaceuticals has not been possible.

[027] We conducted a study using the myoblast cell culture line C2C12 which displays endogenous IGF-1 production that can be reduced by tumor necrosis factor alpha (TNFa). This *in vitro* model mimics and is used to measure the deleterious effects of inflammation, infection and altered immunity, muscle degradation and cachexia. When these cells were treated with various Maca extracts, there was a

rapid upregulation of IGF-1 gene expression, using real time reverse-transcriptase polymerase chain reaction (RT-PCR). This was apparent within 4 hours and persisted through to at least 24 hours with a single exposure. It was also noted that the magnitude of this increase in IGF-1 gene expression was greatest with an aqueous extract of Maca and while evident with a methanol extract, it was of reduced size and duration.

[028] This ability of the Maca extract RNI 510 herein described to promote IGF-1 gene expression defines its uses.

[029] In fetal growth, RNI 510 prevents altitude-associated fetal growth restriction. Hypoxia as experienced at high altitude is a direct suppressor of IGF-1 production and IGF-1 is a direct determinant of fetal growth. Compromised fetal growth caused by infection, elevated cytokine production or poor nutrition are also associated with compromised IGF production as it is a critical pathway for regulating fetal growth. Thus the elevation of endogenous IGF-1 would ameliorate associated conditions.

[030] Fertility in both males and females is regulated by the local production of IGF-1. Given that ingestion of Maca can improve indices of fertility and prevent hypoxia-induced deficits in fertility, the enhanced IGF-1 promoting components of RNI 510 extract represent an innovative manner to stimulate IGF-1 dependent regulation of male and female fertility.

[031] Cartilage is an example of a tissue source that produces IGF locally as an anabolic factor. RNI 510 promotes cartilage growth, deposition of major matrix components and repair by preventing degradation induced by pro-inflammatory cytokines. As no therapy currently exists which directly promotes cartilage

anabolism, IGF promoting methods and compositions contained in this embodiment represent a major innovation in the therapy of conditions such as osteoarthritis.

[032] As we age, there is a natural decline of IGF gene expression and production, which can be exacerbated by underlying inflammation. Those individuals with low IGF levels, such as the elderly, are at greater risk for restricted mobility, dysfunction and compromised quality of life. IGF not only promotes cartilage anabolism but also promotes strength, mass and tone of the surrounding skeletal muscle. This in turn provides greater strength and flexibility to the joint. Thus age-related declines in mobility, arthritis and quality of life can be treated with the composition RNI 510 described herein through its ability to promote endogenous IGF production, effectively reversing the decline associated with aging.

[033] Increased muscle mass, strength and tone are anabolic characteristics of IGF-1 and thus RNI 510. As anabolic steroids have detrimental side-effects, RNI 510 offers an alternative approach to enhance endogenous IGF-1 production as an anabolic stimulus devoid of the complications associated with pharmacological agents and thus a significant advantage.

[034] In diabetes, glucose regulation is impaired by low insulin levels or depressed tissue responsiveness to insulin. By promoting the production of insulin-like growth factor (IGF) at the local (endogenous) level, RNI 510 improves glucose regulation and offers an alternative which could be beneficial in the treatment of diabetes. .

[035] RNI 510 through its actions on IGF-1 can promote weight loss and a leaner muscle mass by regulating glucose levels. To date weight management programs

seeking to promote a leaner muscle mass have not been able to use approaches that enhance IGF-1 expression.

[036] In young animals, humans, pigs, poultry, and ectotherms (i.e. fish), growth rate is determined by the production of IGF-1. Growth may be stunted by infection, inflammation, stress or diet which in turn suppresses the production of IGF-1. By enhancing IGF-1 production, RNI 510 and related extracts can promote growth. We conducted a study in fish farming where environmental influences have a large impact on growth and survival. We have shown that Maca and polar but not lipidic extracts promote fish growth, anabolism and enhanced survival.

[037] In this study we noted an increase in the feed conversion ratio, where for each unit of food ingested, there was a corresponding 20% increase fish size, indicating anabolism.

[038] Aging is associated with a reduction of IGF-1 expression and production, and this contributes to a reduced quality of life. Ingestion of Maca is known for its promotion of a feeling vitality that can be explained by enhanced IGF-1 production.

[039] Cachexia or muscle wasting can occur in numerous conditions of infection, aging, altered immunity and is mediated at the tissue level by suppression of IGF production. By enhancing muscle production of IGF these deleterious effects can be negated.

[040] Growth hormone is a stimulant for hepatic production of IGF-1 but is a poor regulator of tissue production of IGF-1. Individuals that are not responsive to growth hormone have depressed IGF-1 levels. RNI 510, by enhancing IGF-1 production at

the tissue level independently of growth hormone, offers a new approach to managing this condition.

[041] Children suffering from persistent inflammation and cytokine production, as in inflammatory bowel disease or chronic renal inflammation, are growth impaired and display reduced levels of IGF-1, in part because of the ability of cytokines to suppress IGF-1 gene expression. By enhancing the endogenous production of IGF-1, RNI 510 acts as a novel means of treating these individuals and restoring growth rates.

[042] Bone formation and healing is amplified by IGF-1. Young and the elderly are dependent on IGF-1 production for adequate bone density. By increasing IGF-1 production, RNI 510 is an innovative treatment for osteoporosis, enhanced bone growth in childhood and to assist in fracture repair.

[043] Low IGF-1 levels as seen in cirrhosis are associated with adverse outcomes and are considered pathogenic to the complications of cirrhosis. Resistance to the stimulatory effects of growth hormone is common. By providing an alternative approach to raising IGF levels, RNI 510 offers an innovative approach to managing cirrhosis and other conditions of hepatic damage such as toxic substance-induced damage and gastrointestinal disease.

[044] Deficits in cognition associated with aging are associated with reduced IGF-1 levels. By elevating IGF-1 production, RNI 510 may pose a unique means of treating the cognitive problems of the elderly, including Alzheimer's disease.

[045] Joint, muscle and tissue injury is associated with enhanced local pro-inflammatory mediators including matrix metalloproteases, which promote tissue

degradative processes and diminished IGF-1 production. These can limit the repair process and complete healing. By stimulating the production of IGF-1, RNI 510 can promote repair and limit degradative processes to facilitate a more effective and rapid repair of injuries.

[046] Pro-inflammatory cytokines, enzymes and processes promote catabolic events and suppress IGF-1 production. Thus, IGF-promoting RNI 510 in combination with antioxidants or redox based inhibitors of pro-inflammatory transcription, such as seen with Uncaria species, curcumin, or green tea catechins, would have enhanced benefit.

[047] Considering the known effects of zinc, calcium and dietary proteins on bone and formation and strength, the combination of these agents with RNI 510 would be expected to produce a more effective treatment for disorders of musclo-skeletal system.

## CLAIMS

What is claimed is:

1. A method for extracting or concentrating the polar constituents from plants of the family Brassicaceae, comprising:

Collecting plant material from the Family Brassicaceae;

Increasing surface area of said material through mechanical manipulation;

Exposing said material to steam;

Collecting and condensing the steam into a liquid; and,

Evaporating the liquid to resolve the polar components as an extract.

2. The method of claim 1 wherein the plant material is of the genus Lepidium and the preferred parts used include the hypocotyls or roots.

3. The method in claim 1 wherein the mechanical means used to increase the surface area of the plant material may include chopping, grinding, pureeing or macerating and the final material may take the form of pieces, granules, a macerate, a puree or a powder.

4. The method in claim 1 wherein the plant material is spread upon mesh sheets or contained in mesh such that steam can pass through said material and said material exposed to steam in a controlled environment, such as a distillation vessel, for a period of between 15 minutes and eight hours

5. The method in claim 1 wherein the steam is first collected and then condensed in a collector through temperature variation into a liquid.

6. The method in claim 1 wherein the condensed steam is deplete of its aqueous components through evaporation, heating, vacuum drying, lyophilization or other mechanical methods.

7. The extract of claim 1 containing concentrated polar components and reduced lipid components, lignans and starches relative to the parent material.

8. The extract of claim 1 comprised of a pharmacological dose unit which enhances the expression of the gene coding for insulin-like growth factor (IGF) and production of IGF locally in tissues and systemically.

9. A pharmacological dosage unit in claim 8 which increases IGF and negates compromises in fetal growth due to infection, hypoxia, high altitude and poor nutrition.

10. A pharmacological dosage unit in claim 8 which stimulates gonadal production of IGF-1 and promotes indices of male and female fertility.

11. A pharmacological dosage unit in claim 8 which increases IGF and negates the degradation of cartilage, promotes tissue repair and improves joint mobility and function in the elderly or those suffering from osteoarthritis.

12. A pharmacological dosage unit in claim 8 which increases IGF and promotes muscle growth, strength and tone.

13. A pharmacological dosage unit in claim 8 which increases IGF and regulates blood glucose levels and is suitable for use in diabetes.

14. A pharmacological dosage unit in claim 8 which increases IGF and reduces the urge to snack and so can be used to promote weight loss or leaner body mass

15. A pharmacological dosage unit in claim 8 which increases IGF and promotes the growth of ectotherms including fish, as well as pigs, poultry and other farmed animals when included in meal formulations.

16. A pharmacological dosage unit in claim 8 which increases IGF and enhances the survivability of fish, pigs and poultry.

17. A pharmacological dosage unit in claim 8 which increases IGF levels whose depletion is associated with the aging process..

18. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes fertility in individuals with normal levels of sex steroids, gonadotrophins, prolactin and thyroid hormone.

19. A pharmacological dosage unit in claim 8 which increases IGF levels and is effective in the reduction or prevention of miscarriages.

20. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes weight gain in cachexia.

21. A pharmacological dosage unit in claim 8 which increases IGF levels in those individuals that exhibit growth hormone insensitivity.

22. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes growth in children whose growth is compromised by a background chronic infection, inflammation or state of enhanced production of cytokines.

23. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes bone formation in the young, fracture healing, osteoporosis and the aged.

24. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes liver function in subjects with liver disease, gastrointestinal disease or burns.

25. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes cognitive function in the elderly.

26. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes muscle and joint repair after injury.

27. A pharmacological dosage unit in claim 8 which increases IGF levels and is combined with anti-inflammatory agents including cyclo-oxygenase inhibitors, green tea catechins, cat's claw, cucumerin, and or antioxidants to enhance therapeutic benefit in states of inflammation, infection and imbalanced immunity.

28. A pharmacological dosage unit in claim 8 which increases IGF levels and promotes muscle and bone anabolism and may be combined with zinc, proteins and calcium to further enhance benefits.

## ABSTRACT OF DISCLOSURE

An extract and procedure for extracting the polar constituents from plants of the Family Brassicaceae, specifically but not limited to the genus Lepidium. The extract has increased polar and decreased lipidic constituent concentrations with the ability to promote the expression and production of insulin-like growth factor. The extract makes the product more amenable to use in preparations for use in conditions that are associated with reduced insulin-like growth factor levels in humans and animals, including growth, muscle mass, strength and repair, arthritis, bone formation and osteoporosis, dysmobility in the elderly, improved liver and gastrointestinal function, cachexia, fertility, fetal and neonatal growth restriction, aquaculture and animal farming.

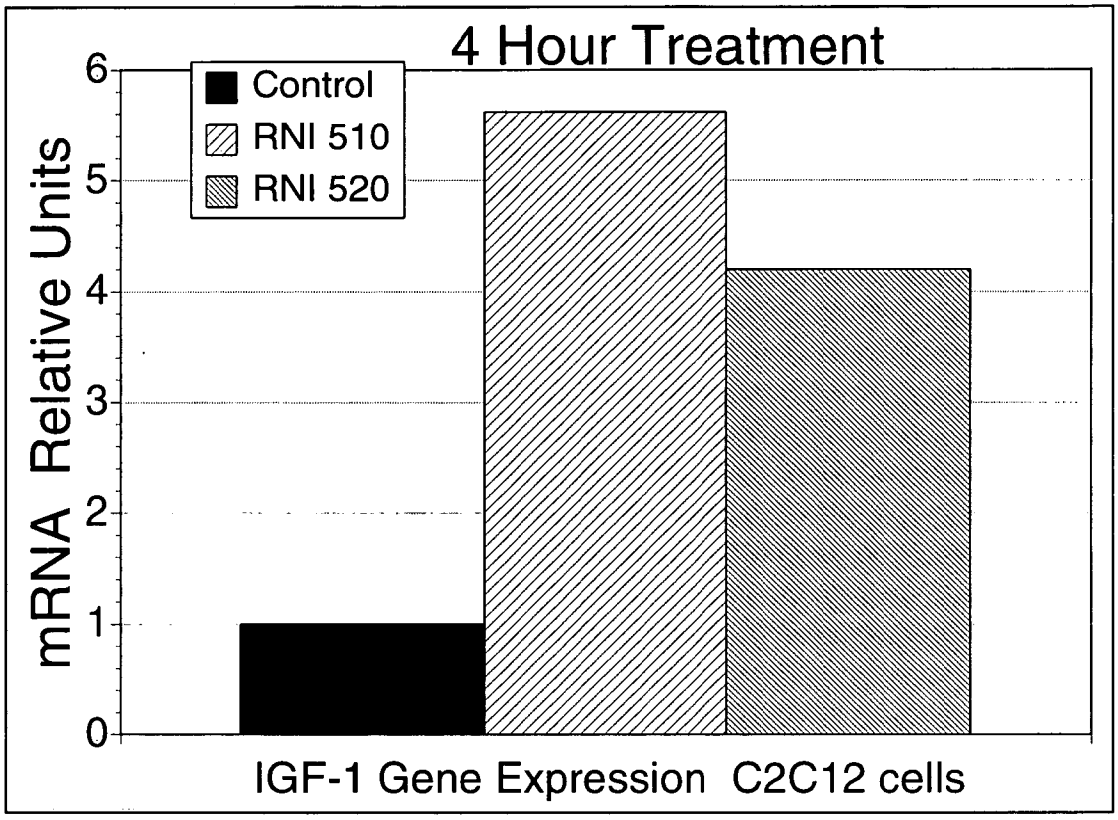
4 Hour Treatment
Control
RNI 510
RNI 520
mRNA Relative Units
0
1
2
3
4
5
6
IGF-1 Gene Expression C2C12 cells

**Figure 1**

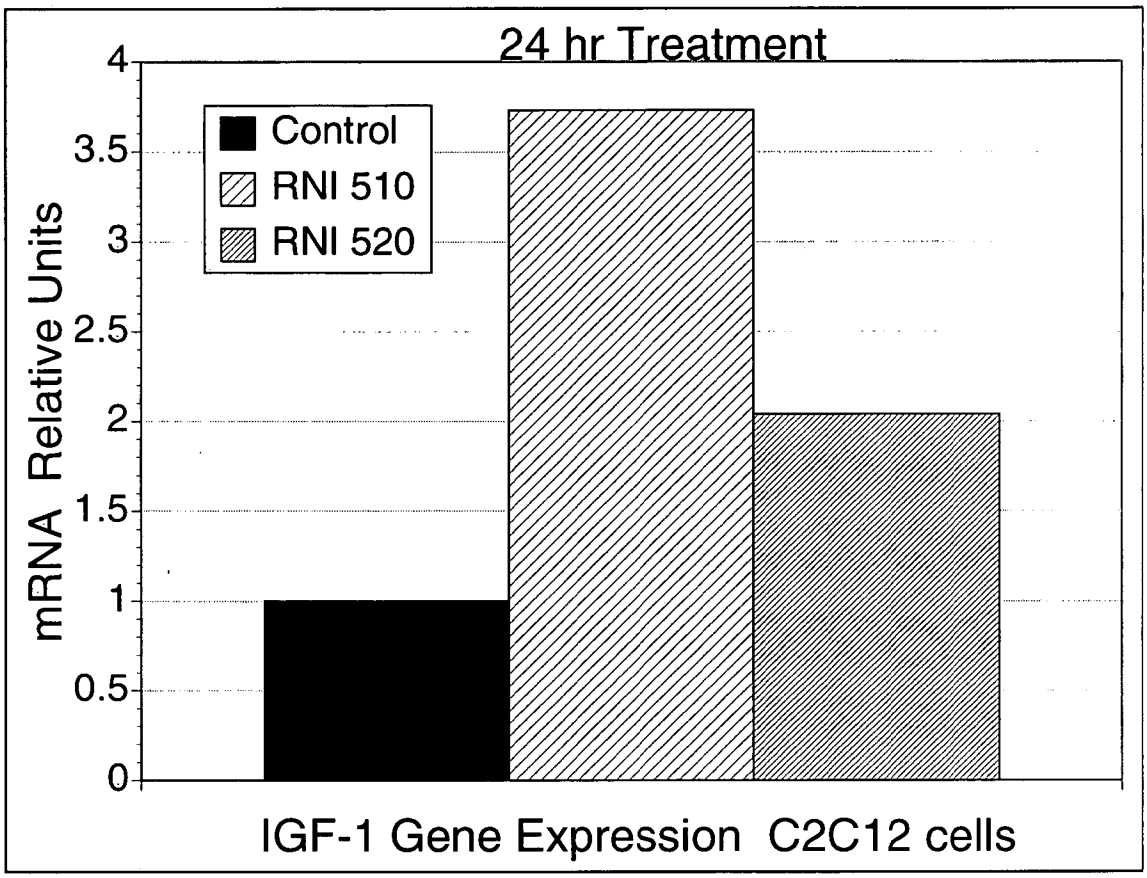
24 hr Treatment
Control
RNI 510
RNI 520
mRNA Relative Units
4
3.5
3
2.5
2
1.5
1
0.5
0
IGF-1 Gene Expression C2C12 cells

**Figure 2**

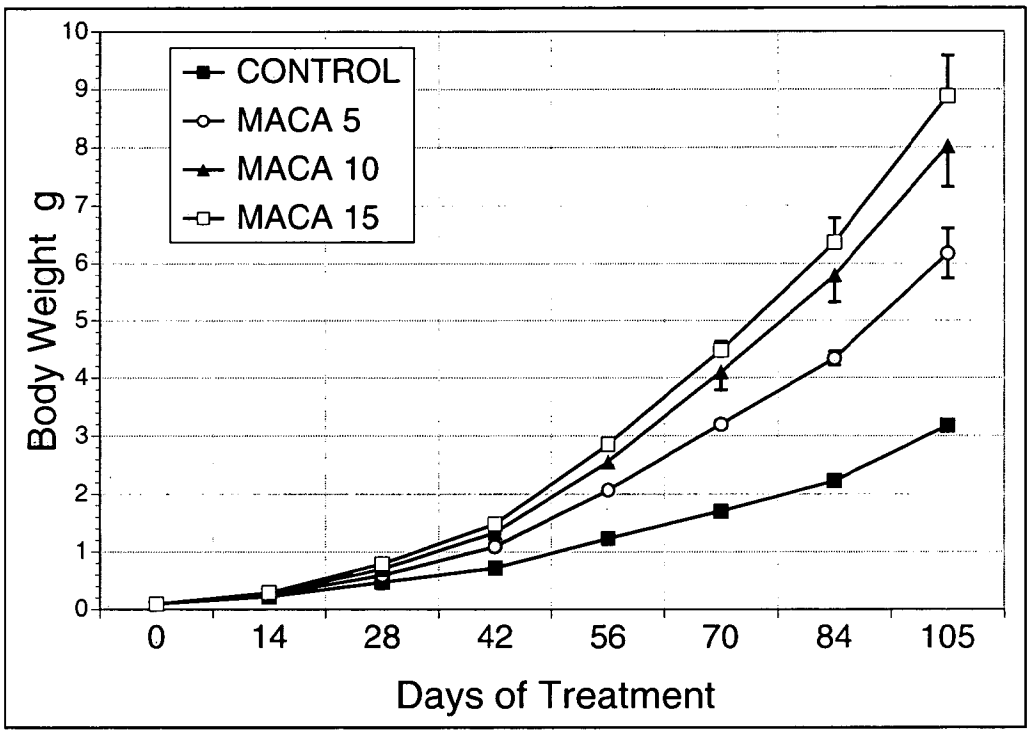
CONTROL
MACA 5
MACA 10
MACA 15
Body Weight g
0
1
2
3
4
5
6
7
8
9
10
0
14
28
42
56
70
84
105
Days of Treatment

**Figure 3**

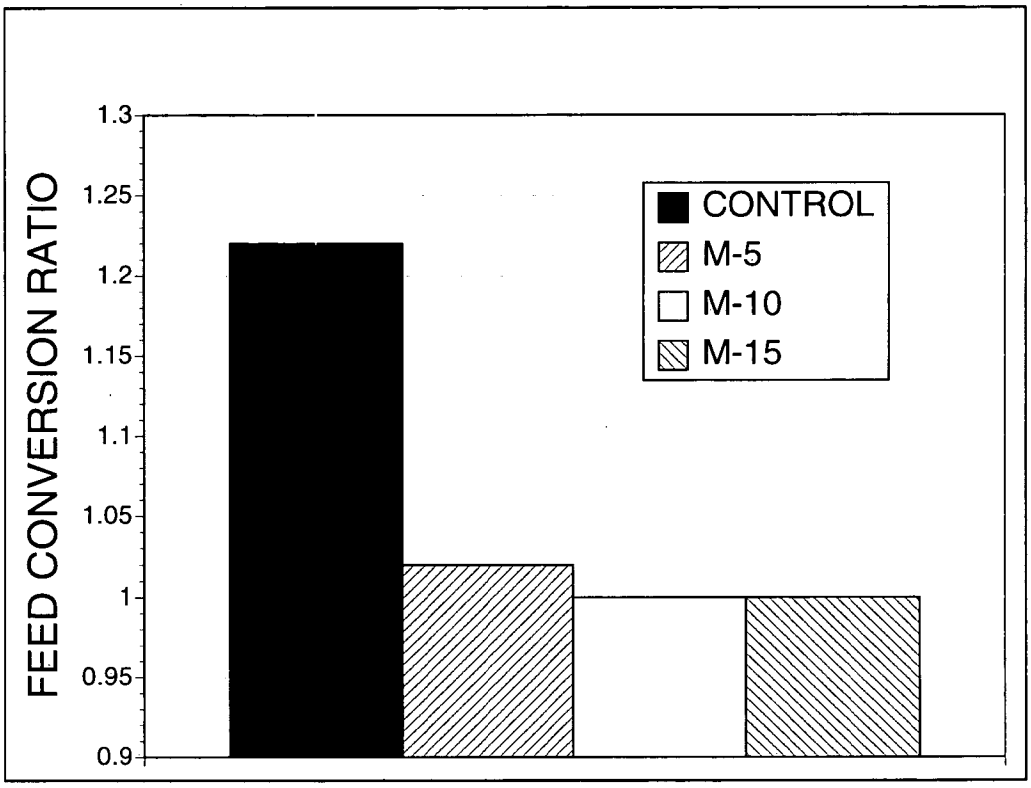
FEED CONVERSION RATIO
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
CONTROL
M-5
M-10
M-15

Figure 4

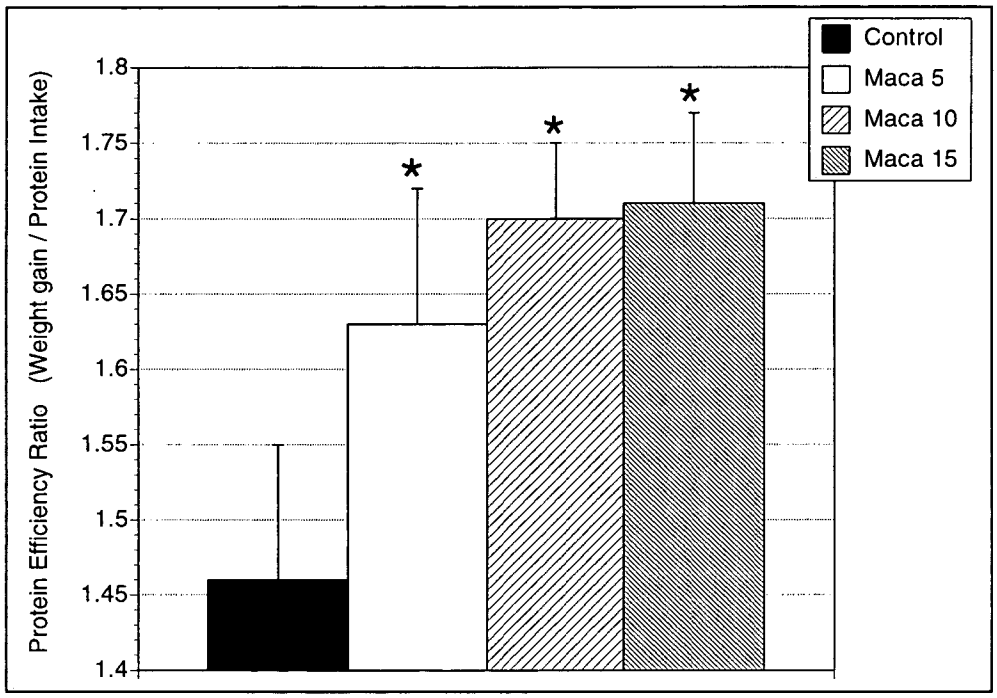
Protein Efficiency Ratio (Weight gain / Protein Intake)
1.8
1.75
1.7
1.65
1.6
1.55
1.5
1.45
1.4
*
*
*
Control
Maca 5
Maca 10
Maca 15

Figure 5

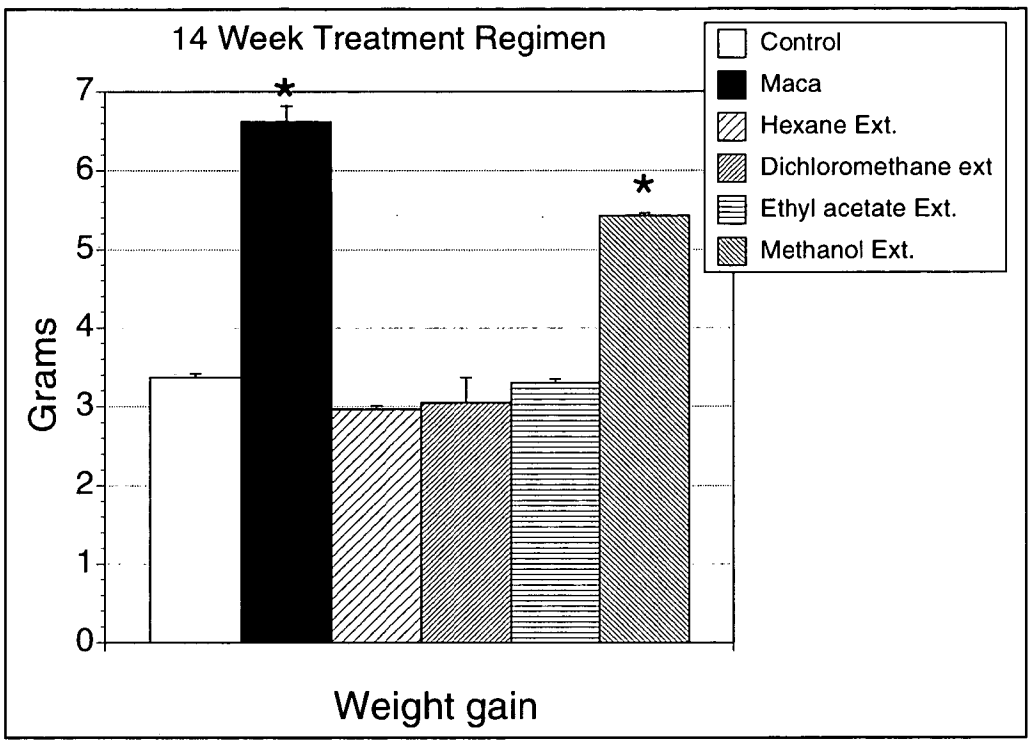
14 Week Treatment Regimen
Control
Maca
Hexane Ext.
Dichloromethane ext
Ethyl acetate Ext.
Methanol Ext.
Grams
0
1
2
3
4
5
6
7
*
*
Weight gain

**Figure 6**

PTO/SB/01A (08-03)
Approved for use through 06/30/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE


# DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

| Title of Invention | METHODS AND COMPOSITIONS TO ENHANCE ENDOGENOUS IGF PRODUCTION AND THEIR USES |
|---|---|

As the below named inventor(s), I/we declare that:

This declaration is directed to:

☑ The attached application, or

☐ Application No. _____, filed on _____,

☐ as amended on _____(if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

All statements made herein of my/own knowledge are true, all statements made herein on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)

Inventor one: PAUL JOSEPH BOBROWSKI

Signature: _____ Citizen of: USA

Inventor two: _____

Signature: _____ Citizen of: _____

Inventor three: _____

Signature: _____ Citizen of: _____

Inventor four: _____

Signature: _____ Citizen of: _____

☐ Additional inventors or a legal representative are being named on _____additional form(s) attached hereto.